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Avian Influenza A (H5N1) in 10 Patients in Vietnam

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ABSTRACT

BACKGROUND

Recent outbreaks of avian influenza A (H5N1) in poultry throughout Asia have had major economic and health repercussions. Human infections with this virus were identified in Vietnam in January 2004.

METHODS

We report the clinical features and preliminary epidemiologic findings among 10 patients with confirmed cases of avian influenza A (H5N1) who presented to hospitals in Ho Chi Minh City and Hanoi, Vietnam, in December 2003 and January 2004.

RESULTS

In all 10 cases, the diagnosis of influenza A (H5N1) was confirmed by means of viral culture or reverse transcriptase–polymerase chain reaction with primers specific for H5 and N1. None of the 10 patients (mean age, 13.7 years) had preexisting medical conditions. Nine of them had a clear history of direct contact with poultry (median time before onset of illness, three days). All patients presented with fever (temperature, 38.5 to 40.0°C), respiratory symptoms, and clinically significant lymphopenia (median lymphocyte count, 700 per cubic millimeter). The median platelet count was 75,500 per cubic millimeter. Seven patients had diarrhea. In all patients, there were marked abnormalities on chest radiography. There was no definitive evidence of human-to-human transmission. Eight patients died, one patient has recovered, and one is recovering.

CONCLUSIONS

Influenza A (H5N1) infection, characterized by fever, respiratory symptoms, and lymphopenia, carries a high risk of death. Although in all 10 cases the infection appears to have been acquired directly from infected poultry, the potential exists for genetic reassortment with human influenzaviruses and the evolution of human-to-human transmission. Containment of influenza A (H5N1) in poultry throughout Asia is therefore urgently required.

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Influenza A virus infects a variety of animals, including humans and birds.[1] Although the natural reservoir for all known subtypes of influenza A (hemagglutinins H1 through H15 and neuraminidases N1 through N9) is wild waterfowl, only three subtypes are currently circulating among humans (H1N1, H1N2, and H3N2). However, during the past few years, several subtypes of avian influenza A have been shown to cross the species barrier and infect humans. During an outbreak of a highly pathogenic influenza A (H5N1) virus among poultry in Hong Kong in 1997, 6 of 18 people with confirmed infection died.[2] After this outbreak, prevention policies and early detection strategies were put into place, and no new cases of H5N1 were detected in Hong Kong until February 2003, when two cases were reported, one of which resulted in death.[1] Possibly as a result of heightened surveillance, avian influenza A (H9N2) viruses were also isolated from children in Hong Kong in 1999, but this infection resulted in only mild, self-limiting illnesses.[3,4]

A total of 89 human infections with influenza A (H7N7), including 1 resulting in the death of a Dutch veterinarian, occurred during the extensive outbreak in 2003 that decimated the Dutch poultry industry.[5,6] During late 2003 and early 2004, there were reports of large outbreaks of H5N1 among poultry throughout Asia (including South Korea, Japan, Indonesia, Vietnam, Thailand, Laos, Cambodia, and China). In January 2004, there was confirmation that influenza A (H5N1) virus had been isolated from patients who had died of a respiratory illness in Hanoi and Ho Chi Minh City, Vietnam. We report the epidemiologic, clinical, and radiographic features in 10 patients with confirmed influenza A (H5N1) infection.

METHODS

PATIENTS

We identified 10 patients in Vietnam with avian influenza A (H5N1) virus infection as confirmed by culture or reverse-transcriptase polymerase chain reaction (RT-PCR). Four of the patients (Patients 1, 2, 3, and 4) were admitted to the National Hospital for Pediatrics in Hanoi between December 27, 2003, and January 14, 2004. The other six patients (Patients 5, 6, 7, 8, 9, and 10) were admitted to the Hospital for Tropical Diseases in Ho Chi Minh City between January 20 and January 30, 2004. Epidemiologic data were collected through interviews of the patients and their relatives. Data on vital signs, physical findings, and routine laboratory tests were obtained by means of a retrospective review of the hospital records (for Patients 1, 2, 3, and 4) and from prospectively recorded case notes (for Patients 5, 6, 7, 8, 9, and 10).

RADIOLOGIC ASSESSMENT

Chest radiographs were obtained in all patients during hospitalization and were reviewed by experienced clinicians. The radiologic findings were categorized with attention to unilateral or bilateral changes; focal, lobar, or patchy consolidation; airspace infiltrates; air bronchograms; pleural effusions; and volume loss with or without shift.

MICROBIOLOGIC EVALUATION

Blood cultures were obtained from all patients on admission to the hospital. Throat and nasal swabs and, when appropriate, tracheal aspirates were collected in viral transport medium (Minimum Essential Medium Eagle with Hanks' salts, supplemented with 0.5 percent gelatin and antibiotics [Sigma-Aldrich]) or phosphate-buffered saline and stored at –80°C. In the four patients in Hanoi, swabs were obtained and stored in a collection and transport system for viruses and chlamydiae (Multi-Microbe Medium Collection and Transport System [M4RT], Remel). Virus was cultured in monolayers of Madin–Darby canine kidney cells. Isolated virus was identified by means of immunofluorescence and hemagglutination-inhibition assays, as previously described.[1] Rapid influenza tests (Capillia Flu A/B Test [Nippon Becton Dickinson] or QuickVue [Quidel]) were used according to the manufacturers' instructions to test nose and throat swabs from six patients.

RNA EXTRACTION AND RT-PCR

RNA was extracted from 140 μl of nasal and throat swab samples in phosphate-buffered saline or viral transport medium with the use of a viral RNA kit (QIAamp, Qiagen) and a double elution with 2× 40 μl of buffer; 5 μl of the RNA extract was analyzed in the RT-PCR assay. Reverse-transcriptase reactions contained 2 μl of 5× first-strand buffer, 2.5 μM random hexamer primers (Roche Diagnostics), 40 units of RNase inhibitor (RNase OUT, Invitrogen), and 1 μl (200 units) of reverse transcriptase (Superscript II, Invitrogen). Reverse-transcriptase reactions were performed at room temperature for 10 minutes, then at 42°C for 30 minutes and at 70°C for 5 min-

utes; 2 μl of the cDNA was used for amplification in the PCR assays. The reactions (total volume of 25 μl) contained 2.5 μl of 10× PCR Gold buffer, 2.5 mM magnesium chloride, 0.4 mM deoxynucleoside triphosphates (Roche Diagnostics), 0.8 mM of each of the two primers, and 0.5 unit of AmpliTaq Gold DNA polymerase (Applied Biosystems).

The samples from Patients 1, 2, 3, and 4 were tested with the primer set for the H5 gene (forward primer H5-1: GCCATTCCACAACATACACCC; reverse primer H5-2: TAAATTCTCTATCCTCCTTTCCAA) with an expected product size of 358 bp,[2] and the primer set for the N1 gene (forward primer N1-1: TTGCTTGGTCAGCAAGTGCA; reverse primer N1-2: TCTGTCCATCCATTAGGATCC) with an expected product size of 615 bp.[7] Thermal cycling for these reactions was performed under the following conditions: 94°C for 3 minutes; 40 cycles of 94°C for 30 seconds, 45°C (for H5) or 55°C (for N1) for 30 seconds, and 72°C for 1 minute; then 72°C for 7 minutes.

The samples from Patients 5, 6, 7, 8, 9, and 10 were tested with two different primer sets that are specific for the influenza A subtype H5 gene: primer pair H5-1 and H5-2 (as described above) and primer pair H5b (forward primer H5/515: CATACCCAACAATAAAGAGG; and reverse primer H5/1220: GTGTTCATTTTGTTAATGAT, with an expected product size of 708 bp). For samples from these six patients, the influenza A N1 gene was amplified with the primers described above, except that there was a modification in the N1-1 primer: TTGCTTGGTCAGCAAGTGCT. All patients were tested for the H1 subtype of influenza A (with the forward primer H1: AGCAAAAGCAGGGGAAAATAA and the reverse primer H1: GCTATTTCTGGGGTGAATCT; expected size of the PCR product, 729 bp) and the H3 subtype of influenza A (with the forward primer H3: AGCAAAAGCAGGGGATAATTC and the reverse primer H3: TGCCTGAAACCGTACCAACC; expected product size, 1143 bp).

Thermal cycling for all amplifications, except for that of the influenza A N1 gene fragment, was 95°C for 10 minutes (preamplification hot start); 10 cycles of 95°C for 30 seconds, 55°C for 30 seconds (decreased by 1°C per cycle), and 72°C for 1 minute; and 40 cycles of 95°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1 minute. For the N1 gene fragment, thermal cycling conditions were 95°C for 10 minutes; 10 cycles of 95°C for 30 seconds, 60°C for 30 seconds (decreased by 1°C per cycle), and 72°C for 1 minute; and 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute.

Products were analyzed on a 2 percent agarose gel. Precautions for the avoidance of cross-contamination were strictly observed. All samples were obtained and transported in individual sealed bags. The preparation of RT-PCR mixtures, nucleic acid extractions, and amplification and analysis of PCR products were performed in three separate laboratories. Aerosol-resistant filter tips (A.R.T., Molecular BioProducts) were used throughout all laboratory procedures. Negative controls were included during the RNA extraction, reverse transcription, and PCR amplification.

RESULTS

PATIENTS AND CONTACT HISTORY

The mean age of patients (four of whom were female and six male) was 13.7 years (range, 5 to 24); none had any known, clinically significant preexisting medical conditions. Patients 8, 9, and 10 were smokers. The patients were from both rural and urban parts of Vietnam (Fig. 1). Seven of the patients were children attending school. Patients 8, 9, and 10 came from the same district in Lam Dong Province, were from the K'hor ethnic group, and worked as subsistence farmers. There was no contact among the patients before hospitalization.

For eight of the nine patients in whom a history could be obtained, there was clear evidence of either direct handling of poultry (chickens or ducks) or exposure to sick poultry in the week before the onset of illness (Table 1). The median time between exposure and the onset of illness was 3 days (range, 2 to 4) and the median time between the onset of illness and hospitalization was 5.9 days (range, 3 to 8). None of the patients had been involved in the organized culling of poultry.

There were similar illnesses reported in relatives of Patients 1 and 2. Patient 1 became sick on December 25, 2003, was admitted to the hospital on December 27, and died on December 30. Her mother became sick on January 1, 2004, was admitted to the hospital on January 5, and died on January 9. Influenza A (H5N1) infection was confirmed in the mother (who is not included in this report). There was no illness reported in the father or sibling of Patient 1. The seven-year-old sister of Patient 2 reportedly died of a respiratory illness on the day Patient 2 was admitted to the hospital, but no clinical

Figure 1. Map of Vietnam Showing the Reported Cases of Avian Influenza A (H5N1).

details are available. There was no illness reported in the parents or two other siblings of Patient 2. No illness during the preceding two weeks or during hospitalization was reported in any family member or contacts of the other patients.

Infection-control measures were initiated in both hospitals as soon as it was suspected that this illness was caused by influenza A (H5N1). No negative-pressure isolation facilities were available. Patients 1 and 2 were admitted before influenza A (H5N1) was suspected, and therefore their cases were managed with universal precautions but without additional infection-control measures.

CLINICAL AND OTHER FEATURES

The clinical features of the patients with confirmed infection with influenza A (H5N1) virus are summarized in Table 2. All patients presented with fever, shortness of breath, and a cough; in five patients, there was a history of sputum production, and in three of these patients, the sputum was blood-stained. Two patients reported pleuritic pain. Diarrhea was reported in seven of the patients. Bleeding from the nose and gums was noted in one patient on the fourth day of illness. No patient had a sore throat, conjunctivitis, rash, or a runny nose. Physical examination in nine patients revealed fever, rapid respiratory rate (median, 55 breaths per minute; range, 28 to 70), respiratory distress, and crackles on examination of the chest.

LABORATORY ASSESSMENT

The results of the basic laboratory tests performed on admission are shown in Table 3. The median total leukocyte count on admission was 2100 per cubic millimeter (range, 1200 to 3400). The median total lymphocyte count was 700 per cubic millimeter (range, 250 to 1100). The median ratio of CD4-positive cells to CD8-positive cells (measured in Patients 5, 7, 8, 9, and 10) was 0.70 (range, 0.59 to 1.08). The median platelet count was 75,500 per cubic millimeter (range, 45,000 to 174,000). Measurements of alanine aminotransferase and aspartate aminotransferase levels on admission were available in six patients and were elevated in five of them (Patients 1, 4, 5, 6, and 10); one patient (Patient 10) had an elevated serum creatinine concentration on admission. Three patients (Patients 7, 8, and 9) had marked hyperglycemia that developed during hospitalization.

MICROBIOLOGIC ASSESSMENT

Blood cultures were all negative. *Staphylococcus aureus* was isolated from a tracheal aspirate from Patient 1, and *Haemophilus influenzae* from a tracheal aspirate from Patient 2. The diagnosis of influenza A (H5N1) was confirmed through the isolation of the virus from postmortem lung tissue from Patient 1 and throat swabs from Patient 2. In all other patients, the diagnosis was made by means of RT-PCR with the use of primers specific to H5 and N1 in samples

Table 1. Epidemiologic Data.

Patient No.	Location in Vietnam	Occupation	Epidemiologic Information
1	Ha Nam	Student	Family members are farmers who do not keep poultry, but many chickens in neighborhood unexpectedly died in the preceding 2 wk; mother died of influenza A (H5N1) Jan. 9, 2004; father and younger sibling healthy.
2	Nam Dinh	Not available	No information available on exposure to sick poultry; 7-yr-old sister died of acute respiratory illness on Dec. 29, 2003; parents and two other siblings healthy.
3	Bac Ninh	Student	Family members are farmers who kept chickens, which died unexpectedly 5 days before onset of illness; parents and older sibling healthy.
4	Ha Tay	Student	Family members are farmers who kept chickens, which died 2 wk before onset of illness; chickens died in patient's house and neighbors' houses during week before onset of illness; parents and 7 other siblings healthy.
5	Ho Chi Minh City	Student	Patient bought duckling as pet and cared for it in her house for 5 days; duck had diarrhea and died, patient buried it, dug it up a day later and reburied it; both patient and brother handled duck; patient also ate barely cooked eggs (Vietnamese delicacy) 2 days before onset of illness; neighbors kept 40 chickens, but no illness reported in these birds; fever developed in patient 3 days after she bought duck; no other poultry or animals at home; no other household members or relatives sick.
6	Ho Chi Minh City	Student	Frequently attended cockfights, held roosters and chickens; no illness reported in the chickens or in 20 people involved in cockfighting; patient walked through live-poultry market 50 m from house on his way to school.
7	Soc Trang	Student	Extensive exposure, including handling of 10 dead or dying chickens in patient's homestead; father and patient prepared dead chickens for eating (removed feathers, washed, cut meat) 3 days before onset of illness; no other household members or relatives sick; no other poultry or animals at home.
8	Lam Dong	Farmer	Direct handling of 50 chickens, including dead chickens, at home (which was also a restaurant); patient and father prepared chickens for eating; no other household members or relatives sick; no other poultry or animals at home.
9	Lam Dong	Farmer	Direct handling of chickens in patient's homestead 3 days before onset of illness; he prepared dead chickens for eating; no one else in family sick.
10	Lam Dong	Farmer	Direct handling of sick ducks and chickens in patient's home; many sick poultry in the district; no other illness in family.

obtained from nasal and throat swabs. Among the patients hospitalized in Ho Chi Minh City, the results on RT-PCR with the use of the H5b (H5/515F and H5/1220R) primer pair were positive in all six patients tested (Fig. 2), whereas the results of RT-PCR with the H5-1 and H5-2 primer pair were positive in four of the six patients tested. None of the patients' samples were positive with the use of primers specific for influenza H1 or influenza H3. The median duration of illness at the time when RT-PCR confirmed the presence of avian influenza A (H5N1) was 6 days (range, 5 to 12).

Influenza A antigens were detected in two of the six patients who were tested. In one patient (Patient 2), virus was isolated from a sample obtained on day 7 of illness but could not be isolated from a sample obtained on day 15.

RADIOLOGIC ASSESSMENT

All chest radiographs were abnormal on admission to the hospital (Fig. 3). The major abnormalities included extensive infiltration bilaterally, lobar collapse, focal consolidation, and air bronchograms. No pleural effusions were noted. All patients had dramatic worsening of findings on chest radiography during hospitalization. Pneumothorax developed in Patients 2 and 4 while they were receiving mechanical ventilation.

TREATMENT AND OUTCOME

All patients were treated empirically with broad-spectrum antibiotics on admission. Patients 1, 2, 3, and 4 received 5 mg of methylprednisolone per kilogram of body weight per day, and Patients 5, 7, and 8 received 1 to 2 mg of methylprednisolone per kilo-

Table 2. Clinical Characteristics of the Patients on Admission.

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Days between exposure to poultry and onset of illness	—	—	—	—	3	2	3	4	3	3
Days since onset of illness	3	7	7	5	8	6	5	6	5	7
Sex	Female	Male	Male	Female	Female	Male	Female	Male	Male	Male
Age (yr)	12	5	10	8	8	13	16	18	24	23
Cough	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dyspnea	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sputum	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes
Diarrhea	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Rash	No	No	No	No	No	No	No	No	No	No
Myalgia	No	No	No	No	No	No	No	No	No	No
Conjunctivitis	No	No	No	No	No	No	No	No	No	No
Fever	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Temperature (°C)	39.5	38.8	39.0	38.5	38.5	39.6	40.0	40.0	39.5	38.7
Blood pressure (mm Hg)	90/60	112/54	105/80	80/40	104/64	110/70	110/60	100/60	110/60	120/80
Respiratory rate (breaths/min)	65	70	64	60	40	40	40	60	50	28
Crackles	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Wheeze	No	No	No	No	No	Yes	No	No	No	No
Other	Enlarged liver	—	—	Bleeding gums	—	—	—	—	—	—

gram four times a day for one, three, and four days, respectively. Five patients were treated with the neuraminidase inhibitor oseltamivir (35 mg twice daily in Patient 5 and 75 mg twice daily in Patients 7, 8, 9, and 10) for up to five days. Ribavirin was given to Patient 3 (800 mg three times a day) and Patient 4 (400 mg three times a day). The antiviral treatment was started on day 5 of illness in Patients 4, 7, 9, and 10; day 6 in Patients 6 and 8; day 11 in Patient 3; and day 12 in Patient 5. Patients 5, 6, 7, 8, 9, and 10 received ranitidine.

Patient 5 required continuous positive airway pressure with supplemental oxygen for the first seven days after admission but was subsequently weaned from this support and given maintenance therapy with 40 percent oxygen. Patients 1, 2, 3, 4, 6, 7, 8, and 9 required mechanical ventilation during the first 48 hours after admission. In all these patients, there was a dramatic deterioration of gas exchange despite pressure-controlled ventilation, high end-expiratory pressures, and a fraction of inspired oxygen of 1.0. Patients 7 and 8 were also treated with dopamine and norepinephrine for hypotension. Patient 7 had a small gastrointestinal hemorrhage on the third day of hospitalization. Patients 7 and 8 had marked hyperglycemia requiring insulin treatment to normalize the blood glucose levels.

Despite a prolonged, severe illness, Patient 5 survived with no major sequelae. Eight other patients died, and one patient is recovering, for a case fatality rate of 80 percent among patients in our series. The median time to death from the onset of illness was 9 days (range, 6 to 17). Neither during the period when these patients were hospitalized nor subsequently was any illness reported in a health care worker or laboratory staff member.

DISCUSSION

To date, there have been 20 confirmed cases of human infection with influenza A (H5N1) in Vietnam and Thailand; 16 of the infected patients have died. We describe the clinical features of 10 cases of confirmed avian influenza A (H5N1) in patients admitted to referral hospitals in Vietnam.

Table 3. Laboratory Values at Presentation.*

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Hemoglobin (g/dl)	13.4	12.6	12.4	12.3	11.3	13.4	11.9	14.5	15.8	17.6
Leukocyte count (per mm³)	2,100	3,400	2,800	1,900	1,200	2,700	3,000	1,700	1,900	2,100
Lymphocyte count (per mm³)	1,100	710	860	250	300	900	500	500	800	700
Neutrophil count (per mm³)	850	2,410	1,900	780	700	1,300	2,500	1,100	1,100	1,300
Platelet count (per mm³)	45,000	174,000	135,000	91,000	117,000	81,000	70,000	69,000	62,000	62,000
CD4:CD8 ratio	NA	NA	NA	NA	0.71	NA	0.62	0.75	0.59	1.08
ALT level (U/liter)	53.7	NA	NA	265	354	254	47	NA	NA	89
AST level (U/liter)	278	NA	NA	1,217	320	1,058	20	NA	NA	110
Serum creatinine (μmol/liter)	50	64	NA	27	34	14	71	89	43	121
Serum glucose (mmol/liter)	NA	NA	NA	NA	NA	NA	19.0	13.5	11.7	4.9
Oxygen saturation during receipt of 40% oxygen (%)	50	70	86	50	95	85	67	81	80	90
Day of illness on which PCR for H5N1 performed	5	7	9	6	12	6	5	6	5	7
Viral culture	+	+	NA	NA	Pending	Pending	Pending	Pending	Pending	Pending
Influenza antigens	NA	NA	NA	NA	+	–	–	+	–	–
Blood culture	–	–	–	–	–	–		–	–	–
Outcome	Died (day 6)	Died (day 17)	Died (day 14)	Died (day 7)	Recovered	Died (day 9)	Died (day 14)	Died (day 9)	Died (day 6)	Recovering

* Normal ranges are as follows: hemoglobin concentration, 13 to 18 g per deciliter; leukocyte count, 4000 to 11,000 per cubic millimeter; neutrophil count, 2200 to 8250 per cubic millimeter; lymphocyte count, 1500 to 4000 per cubic millimeter; CD4:CD8 ratio, 1.4 to 2.0; platelet count, 150,000 to 400,000 per cubic millimeter; alanine aminotransferase (ALT) level, below 37 U per liter; aspartate aminotransferase (AST) level, below 40 U per liter; serum creatinine concentration, 82 to 106 μmol per liter; and serum glucose concentration, 3.9 to 6.4 mmol per liter. NA denotes not available, a plus sign positive, and a minus sign negative. To convert the values for creatinine to milligrams per deciliter, divide by 88.4. To convert the values for glucose to milligrams per deciliter, divide by 0.05551.

The prominent clinical features on admission were those of a severe influenza syndrome with fever, cough, diarrhea, and shortness of breath. The estimated time between the exposure to poultry and the onset of illness suggests an incubation period of two to four days. Diarrhea was present in 7 of the 10 cases. The most striking laboratory findings were marked lymphopenia and thrombocytopenia with a pronounced inversion of the CD4:CD8 ratio in the five patients in whom it could be measured. A recovery of the lymphocyte count and CD4:CD8 ratio was observed only in the two patients who survived. Liver and renal dysfunction or impaired glycemic control was prominent in six of the patients. The patients were all children or young adults.

These clinical presentations were similar to those in the 1997 outbreak of influenza A (H5N1) in Hong Kong, although diarrhea was a more prominent feature in the Vietnamese patients. In addition, the oldest patient in this series was 24 years old, whereas one patient was 54 years old and one was 60 years old in the 1997 outbreak in Hong Kong.[2] However, the mortality in our series was significantly higher than that in the 1997 outbreak.

Eight of the nine patients from whom a clear history could be taken reported close contact with poultry during the week before the onset of illness. A retrospective study after the Hong Kong outbreak showed that visiting poultry markets before the onset of illness was the only significant risk factor.[8] The contact in six of the current cases involved direct handling of chickens or ducks (holding, killing,

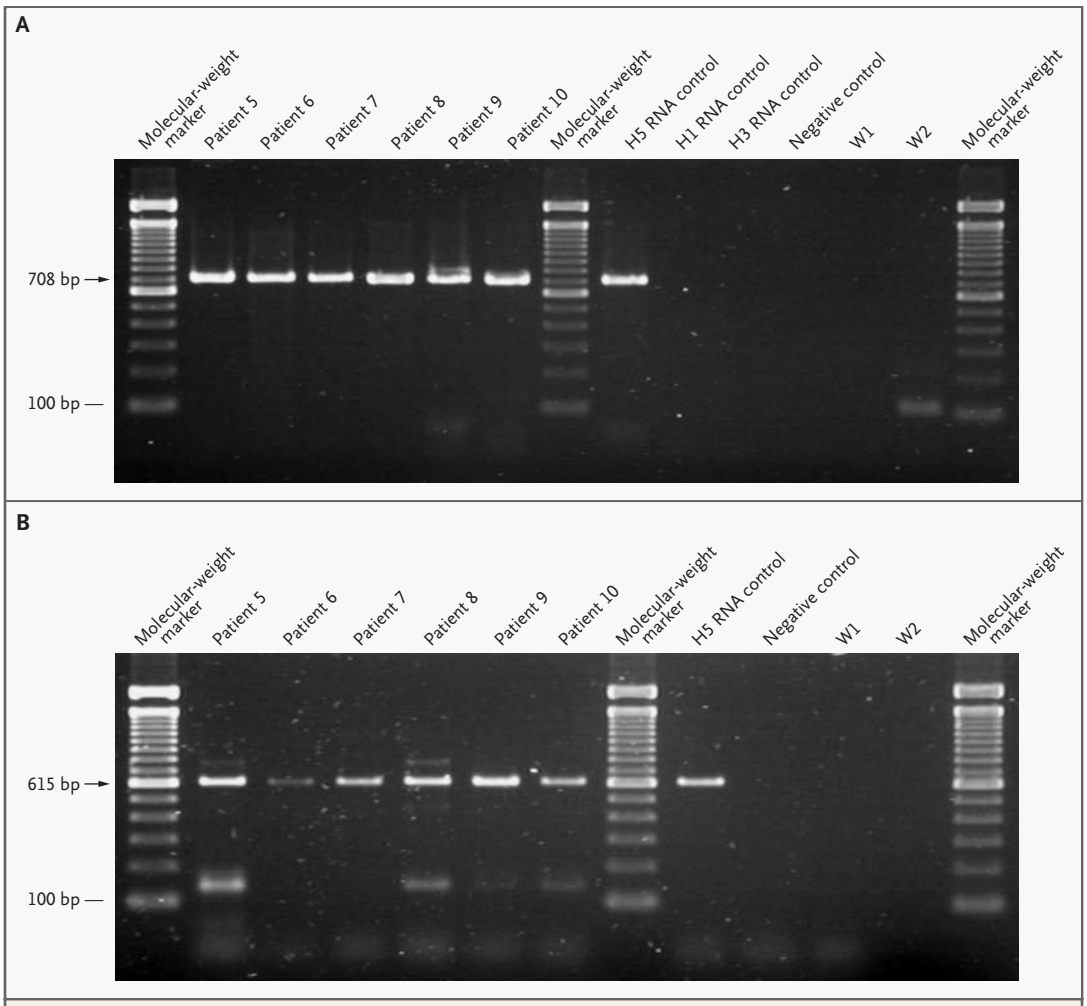

Figure 2. RT-PCR Specific for the H5 Gene of Influenza A (H5N1) Virus, Performed with the Use of the H5b Primers (Panel A), and RT-PCR Specific for the N1 Gene, Performed with the Use of the Modified N1 Primers (Panel B).

Nasal or throat swabs from Patients 5, 6, 7, 8, 9, and 10 were used, as well as an H5 RNA control (A/HK/213/2003 [H5N1]), an H1 RNA control (A/New Caledonia/20/99 [IVR-116; H1N1]), and an H3 RNA control (A/Panama/2007/99 [N1B-41; H3N2]), all cultured in embryonated chicken eggs, and a negative control (RNA-extraction control), W1 (water control for reverse transcriptase), and W2 (water control for PCR).

or defeathering them or preparing them to be eaten) within the patient's home environment or small homesteads nearby, where a relatively small number of chickens were kept. This finding suggests that direct contact is the primary route of bird-to-human transmission. None of the patients were involved in the organized culling of poultry or worked on large poultry farms. This observation, if confirmed, may have important implications for our understanding of the transmission of this virus and potential immunity to it.

The available information on the two family clusters is compatible with bird-to-human transmission from a common source, but there is currently not enough information to rule out limited human-to-human transmission within the family. There was no illness reported in family members of the other eight patients, even though other family members seemed to have had very similar exposure to poultry — for example, the brother of Patient 5 and the fathers of Patients 7 and 8. There is evidence from the 1997 outbreak in Hong Kong that the avian influenza A (H5N1) virus may have been transmitted from human to human but that transmission could

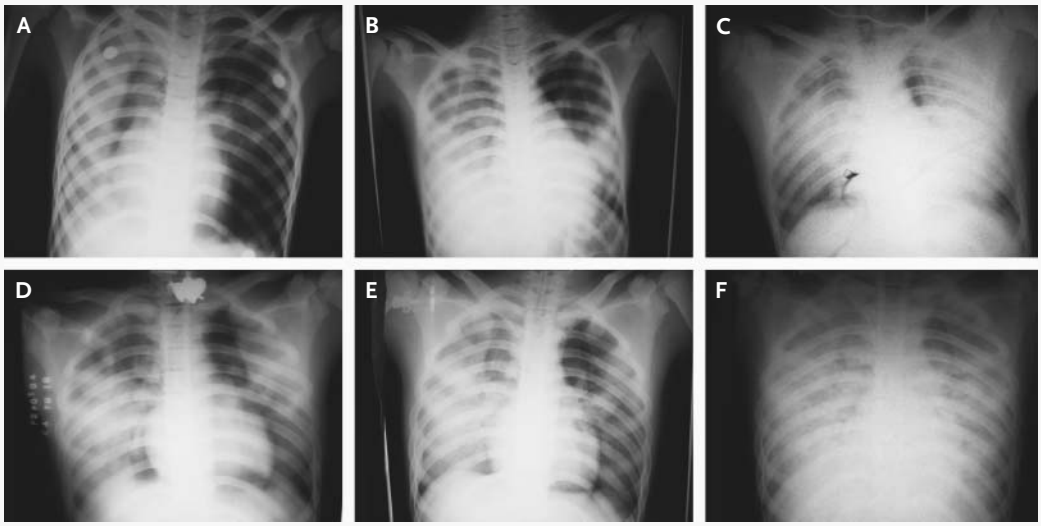

Figure 3. Chest Radiographs.

Radiographs from Patient 5 (Panel A), Patient 7 (Panel B), and Patient 9 (Panel C) show widespread consolidation, collapse, and interstitial shadowing. In Panels D, E, and F, three chest radiographs show the progression in Patient 8 on days 5, 7, and 10 of illness, respectively.

not be sustained among humans.[9,10] The absence of any report to date of a similar illness among the health care workers who cared for these patients, despite the lack of full droplet and respiratory infection-control measures early in the outbreak, is reassuring. We cannot rule out the possibility of mild or subclinical infection in persons exposed to either ill poultry or ill persons. Detailed seroepidemiologic studies of the individual family members, health care workers, and others at risk would be necessary in order to assess whether and to what extent human-to-human transmission has occurred.

Oseltamivir was administered to five of the patients, four of whom died. Treatment with the drug may have been started too late to be effective, although one of the two surviving patients did not start oseltamivir therapy until the 12th day of illness. At that point, she was still antigen-positive and PCR-positive for the virus. There seemed to be no benefit from the oral administration of ribavirin (in Patients 3 and 4). In vitro sensitivity testing of a limited number of strains of influenza A (H5N1) virus isolated from patients in Vietnam have shown that they are resistant to amantadine and rimantadine, so these drugs should not be recommended for treatment. Six of the seven patients who were treated with corticosteroids died. This experience is inadequate to permit the establishment of treatment recommendations with respect to corticosteroids; more aggressive treatments may have been used in patients with a greater severity of illness. Our experience suggests that supportive care may be the only option available. Controlled clinical studies are needed to assess the role of antiviral drugs and corticosteroids in the treatment of influenza A (H5N1) virus infections.

Rapid testing for influenza antigens in a small number of patients on admission was less sensitive than RT-PCR for the diagnosis of influenza A (H5N1). Our experience in this small number of cases suggests that the low sensitivity of the rapid diagnostic tests for influenza may limit their usefulness for the reliable detection of influenza A (H5N1) in humans, especially if patients present relatively late in the course of illness and if other strains of influenza A are circulating simultaneously. The H5b primer pair yielded positive RT-PCR results in all six patients tested in this small series, as compared with positive results in four of six with the use of the H5-1 and H5-2 primers on the same samples. The N1 primers used resulted in nonspecific RT-PCR products and required modification to yield specific results. Further evaluation of the two H5 primer systems is being undertaken. The sensitivity of the RT-PCR methods, which were designed for the identification of influenza A (H5N1) virus from cul-

ture, is unknown, and we urgently need new, properly evaluated, sensitive diagnostic tests.

The clinical findings (fever, cough, diarrhea, shortness of breath, rapid respiratory rate, lymphopenia, and abnormalities on chest radiography) and a history of close contact with poultry may be more helpful in identifying patients with influenza A (H5N1) infection than the results on rapid diagnostic tests for influenza. We do not know whether the clinical syndrome described on the basis of these 10 patients is representative of the true clinical spectrum of the disease, since each of these patients was admitted to a referral hospital. The extent of mildly symptomatic disease in the community remains unknown. Increasing the availability of serologic tests, molecular diagnostic procedures, and viral culture throughout Asia would help considerably.

As documented in Hong Kong in 1997 and 2003, and now in Vietnam and Thailand, the avian influenza A (H5N1) virus clearly has the ability to jump between species and cause devastating illness in humans. Widespread efforts to control the poultry outbreak and increased surveillance among poultry and humans should therefore be our highest priority. It is reassuring that to date there has been no evidence of efficient human-to-human transmission of influenza A (H5N1) virus in either the 1997 or the 2004 outbreak. However, the continued circulation of virulent avian influenza A (H5N1) virus increases the possibility of the reassortment of this virus with other circulating human influenza A viruses and increases the threat of a global influenza pandemic.

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APPENDIX

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